ANTIPOLLEN SERUM FOR STANDARDIZATION OF POLLEN ANTIGEN

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The work[1] on standardization of pollen extract is based either on the weight of the pollen or on a chemical analysis of the extract for its nitrogen content. These methods of standardization do not determine the antigenic strength of the extract; for, while the nitrogen content is an indicator of the amount of pollen protein in the extract, both of the methods fail to show whether the protein of the pollen is active antigenically or whether it is inert.

It seemed, therefore, that if the antigen could be standardized against antipollen serum by immunologic methods, the active antigenic power of the extract might be definitely determined. The carrying out of such tests would, of course, necessitate the employment of a potent serum. Immune rabbit serum has been prepared[2] by injecting the animals subcutaneously in the abdominal region with extracts of plant protein. Regarding this work, Goodale[3] states:

"With reference to the time required for immunization of the rabbits, no exact figures can be given. Sometimes a potent immune serum can be produced after three or four injections, but cases also occur where even after ten injections very little immunity had appeared —at times entirely failed to occur. Apparently the individuality of the animal is a factor."

PREPARATION OF ANTIPOLLEN SERUM

On Jan. 7, 1915, we began the immunization of a series of rabbits, using an extract of whole pollen prepared by adding 3.64 gm. of a mixture of dried pollen consisting of equal parts by weight of the pollens of timothy, red top, June grass, orchard grass, rye, sorrel dock, daisy, maize, ragweed and goldenrod to 182 c.c. of physiologic salt solution, so that the resulting extract contained 20,000 units of pollen per cubic centimeter. The extract was preserved

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[1] Noon: Lancet, 1911, p. 1572. Clowes: Proc. Soc. Exper. Biol. and Med., 1913, 10, p. 70. Koessler: Illinois Med. Jour., 1914, p. 120. Cooke: Laryngoscope, 1915, p. 108. Oppenheimer and Gottlieb: New York Med. Jour., 1915, p. 229. Cooke and Vander Veer: Jour. Immunology, 1916, p. 201.

[2] Mez and Gohlke: Cohn's Beiträge zur Biologie der Pflanzen, 1913. Physiologische-systematische Untersuchungen über die Verwandtschaften der Angio-spermen.

[3] Boston Med. and Surg. Jour., 1915, p. 201.

with 0.25% trikresol and diluted for the injections with sterile saline solution, so that the volume of each injection was 2 c.c., except the last, which was 2.5 c.c. The rabbits were injected every other day with a total of 11 injections, using the following number of units of pollen: 5, 25, 100, 500, 1000, 2000, 5000, 10,000, 20,000, 40,000, and 50,000.

Three rabbits were injected intravenously, 3 intraperitoneally, and 3 subcutaneously. Eight days after the last injection, the animals were anesthetized and bled from the carotid artery. The serum was obtained in a sterile manner, preserved with 0.25% trikresol, and stored in the ice-box at 5 C. After 6 months, the serum became anticomplementary and could no longer be used. Since then, we have preserved all antipollen serum in glycerol in a similar manner to that described in a previous paper;[4] and the serum so preserved has retained its potency and has not exhibited any anticomplementary properties.

TITRATION OF ANTIPOLLEN SERUM

In order to determine the relative potency of the different lots of serum, antibody-content titrations were made, using one-tenth the volume of the classical Wassermann reaction. The technic is indicated in Table 1, which shows the results of titrating antipollen serum from an intravenously-immunized rabbit. The serum was titrated against pollen antigen which was prepared according to the method previously described.[5] Normal rabbit serum was used for controls.

The results of the titrations showed that the serum from the intravenously-immunized and from the intraperitoneally-immunized rabbits

TABLE 1

TITRATION OF ANTI-POLLEN SERUM, FEBRUARY 26, 1915

Number of Tube	Immune Serum 1:10 C.c.	Antigen		10% Complement, C.c.	0.9% Salt Sol., C.c.	Sensitized Erythrocyte Suspension, C.c.	Results
		1:25 C.c.	Gm. of Pollen				
1	0.1	0.1	0.00004	0.1	0.0	0.2	++++
2	0.09	0.1	0.00004	0.1	0.01	0.2	++++
3	0.08	0.1	0.00004	0.1	0.02	0.2	++++
4	0.07	0.1	0.00004	0.1	0.03	0.2	++++
5	0.06	0.1	0.00004	0.1	0.04	0.2	++++
6	0.05	0.1	0.00004	0.1	0.05	0.2	++++
7	0.04	0.1	0.00004	0.1	0.06	0.2	++++
8	0.03	0.1	0.00004	0.1	0.07	0.2	++++
9	0.02	0.1	0.00004	0.1	0.08	0.2	+++
10	0.01	0.1	0.00004	0.1	0.09	0.2	++
11	0.0	0.2	0.00008	0.1	0.0	0.2	—
12	0.2	0.0	0.0	0.1	0.0	0.2	—

This table shows that 0.003 c.c. was the smallest amount of the serum that gave complete fixation of complement.

Citron's standard for the strength of a reaction is used in this table; namely, complete absence of hemolysis is indicated by a 4 plus sign (++++); faint hemolysis is shown by a 3 plus sign (+++); partial hemolysis is represented by a 2 plus sign (++); while a minus sign (—) indicates complete hemolysis.

The mixtures of immune serum, antigen and complement were placed in the ice-box for 15 hours before adding the sensitized erythrocytes. The results were read after 1 hour at 37 C.

[4] Clock and Beard: Jour. Infect. Dis., 1917, 21, p. 404.

[5] Clock: Ibid., 21, p. 387.

was 10 to 15 times more potent than the serum from the subcutaneously-immunized rabbits; the smallest amounts of serum that gave complete fixation of complement being 0.003 c.c., 0.002 c.c., and 0.03 c.c., respectively. Normal rabbit serum, used as controls, failed to show any fixation of complement. In the preparation of all subsequent antipollen serum, the rabbits have been immunized either intravenously or intraperitoneally, and the serum has always proved to be potent.

Each lot of pollen antigen prepared has been accurately standardized against antipollen serum by the complement fixation method. The technic of titrating the antigen was shown in a previous paper.[5] This method of standardization of pollen antigen definitely determines and very accurately measures the active antigenic power of the extract against positive serum of known potency.

SUMMARY

Potent antipollen serum was prepared by immunizing rabbits intravenously or intraperitoneally with an extract of whole pollen.

Eleven injections of an increasing number of units of pollen, ranging from 5 to 50,000, produced immune serum of sufficiently high titer for use as positive serum in standardizing pollen antigen.

Antipollen serum preserved in 50% glycerol retained its potency and did not become anticomplementary after two years.

Antipollen serum, used for standardizing pollen antigen by the complement fixation method, afforded a reliable guide for determining and measuring the active antigenic power of pollen extract.